Available online on 15.06.2019 at http://jddtonline.info

Journal of Drug Delivery and Therapeutics

Open Access to Pharmaceutical and Medical Research

Open Access Research Article

Local Drug Delivery to Bone Joints with Microspheres

Chidambaram Soundrapandian*, Adarsh Pratik Poudyal

Department of Pharmaceutics, Himalayan Pharmacy Institute, Majhitar – 737136, India

ABSTRACT

The concept of local drug delivery in the form of microspheres has potential for use as an alternative to conventional therapy strategies in the treatment of pain and inflammatory symptoms in case of certain bone and joint diseases. Commonly preferred treatment options for these symptoms (such as oral NSAIDs, analgesics, opioid pain medications) need to be frequently administered or applied and additionally, these suffer from multiple limitations. A prolonged release formulation of an NSAID i.e. Diclofenac Sodium, might prevent frequent administrations and improve the therapeutic outcome. In the current research, Diclofenac Sodium (DS)-loaded microspheres were prepared using thermal control and ionic cross-linking techniques. Calcium sulfate hemihydrate ($CaSO_4 \cdot 0.5H_2O$) was used in specific quantities to enhance the self-hardening property of the microspheres. An encapsulation efficiency and loading capacity of up to 90.24% and 61.41% respectively were achieved from the formulations. FTIR analysis indicated no major interactions between the active ingredient and the excipients used in the formulation process. *In-vitro* studies on the biodegradability of the microspheres disclosed that the microspheres showed a slow degradation pattern over time. Release study of the prepared microspheres revealed that the release of DS was prolonged achieving release of drug over a period of up to 11 days. The microspheres seem to fulfill the requisite criteria in-vitro. The results obtained suggest that DS-loaded microspheres have the potential for further investigation and development.

Keywords: Local drug delivery, microspheres, bone and joint diseases, NSAIDs

Article Info: Received 05 May 2019; Review Completed 03 June 2019; Accepted 06 June 2019; Available online 15 June 2019

Cite this article as:

Soundrapandian C, Poudyal AP, Local Drug Delivery to Bone Joints with Microspheres, Journal of Drug Delivery and Therapeutics. 2019; 9(3-s):430-442 http://dx.doi.org/10.22270/jddt.v9i3-s.3043

***Address for Correspondence:**

Chidambaram Soundrapandian, Department of Pharmaceutics, Himalayan Pharmacy Institute, Majhitar – 737136, India

INTRODUCTION

Joint pain and inflammation are the major symptoms of bone and joint diseases and are important considerations in the treatment of these diseases.[1] Such diseases that directly or indirectly lead to these symptoms include but not limited to- Osteoarthritis, Rheumatoid arthritis, Osteoporosis, Paget's disease. The symptoms of these disease can involve functional restrictions but pain and inflammation in the bone joints are the most common and as such the clinical management of these diseases is mostly focused on pain relief and management.[2] However, the conventional treatment strategies commonly adopted for these situations suffer from a several limitations comparable to non-specificity of drug release which directly leads to limitations in their clinical utility, potential side effects of drugs due to requirement of larger doses to elicit a desired therapeutic response as well as fluctuations in drug concentrations which can either lead to good or bad therapeutic response depending upon whether the C_p^{ss} values fall or rise in the therapeutic range.

Current treatment approaches for treating joint pain and inflammations include oral administration of analgesics (acetaminophen), opioid pain medication (tramadol), non-steroidal anti-inflammatory drugs (naproxen, ibuprofen, diclofenac sodium) as well as tricyclic anti-depressants that have been found to be moderately effective in back pain and rheumatoid arthritis.[3,4] Topical application of NSAIDs has also proven helpful in some cases.[5] Invasive procedures include injections of corticosteroids or hyaluronic acid.[6]

Non-steroidal anti-inflammatory drugs (NSAIDs) are one of the most commonly used drugs to ease the pain, inflammation and stiffness that come with arthritis, bursitis and tendinitis.[7] Most NSAIDs are cheap and often among the first medicines prescribed for people with achy joints. They are available to take by mouth or to rub on the skin over painful joints and muscles.

These therapeutic approaches have mainly focused on systemic/conventional administration of drugs. This results in poor bioavailability and biodistribution, prolonged therapy process, frequent intake of drug and low efficacy of the therapy as well as poor retention time at the diseased

site. Moreover, blood perfusion to the bone is less as compared to other organs in the body. Additionally, drugs injected at the diseased sites by intra-articular approaches are often cleared from the site very rapidly ($t_{1/2}$ ~0.1–6 h).[8,9] Topical delivery of drugs has its own set of limitations.[10] To treat the diseases effectively, improvised methods are required, ideally keeping the drug concentrations high at specific sites on the skeletal system without high systemic levels. Buchholz et al., in the 1970s, recognized local drug delivery to the bone as a potential approach to treat bone diseases and conceptualized the skeletal drug delivery system.[11]

Though the development of active targeting delivery systems greatly enhanced the drug concentration at desired disease site after systemic administration, it is still a challenge to control the drug distribution in other organs, especially the high accumulation of nanoparticles in liver or spleen. Local delivery from a drug depot could be a viable alternative. They are especially suitable for the treatment of osteoporotic fractures.[12] One of the most investigated and attractive prospects for delivery of pain medications to the diseased joints is microspheric drug delivery system. This delivery system offers certain advantages over conventional methods of treatment of joint pain and inflammation and also overcomes some of their limitations. Microspheres permit intimate and prolonged contact with the diseased site which has the potential to maximize both the rate as well as the extent of drug absorption. They protect the drug from the degradative environment and enzymes inside the body, reduce frequency of drug intake, reduce side effects, improve bioavailability, reduce fluctuations in plasma drug concentrations, and promote controlled release of the medication.[13]

Ethyl cellulose (EC) and Sodium Carboxymethyl cellulose (Na-CMC) have been studied as one of the materials for controlled release of NSAIDs.[14,15]

In the current investigation, formulation of DS releasing polymeric microspheres of Ethyl cellulose (EC) and Sodium Carboxymethyl cellulose (Na-CMC) was attempted. The DS-loaded EC and Na-CMC microspheres were prepared by thermal control and ionic cross-linking techniques with varying polymer quantities and with the incorporation of specific quantities of ($CaSO_4$). Several properties of the prepared microspheres were studied by suitable evaluation parameters namely drug encapsulation efficiency and loading capacity, yield, particle size analysis, micromeritic properties, moisture loss, buoyancy, compatibility studies by FTIR Analysis, surface morphology by SEM Analysis, in-vitro biodegradability and drug release pattern.

MATERIALS AND METHODS

Materials: Diclofenac Sodium was procured from Central Drug House (P) Ltd., New Delhi. Ethyl Cellulose and Sodium Carboxymethylcellulose were obtained from SDFCL, Mumbai. Cyclohexane and Sodium Alginate were acquired from Universal Laboratories Pvt. Ltd., Mumbai and Loba Chemie Pvt. Ltd. respectively. n-Hexane and Calcium chloride were obtained from SDFCL, Mumbai. All chemicals used in the research are of analytical grade and were used as received.

Methods:

Solubility testing of drug: This study was performed by taking an excess quantity of drug sample in specified quantities of different solvents- water, ethanol, methanol, acetone, PBS pH 7.4 and cyclohexane.[16,17]

Melting point of drug: This study was carried out by Thiele tube method to indicate the purity of the sample. Adequate quantity of drug was filled in a glass capillary tube and the tube was attached to a thermometer by using a string or rubber band. The thermometer was dipped in fresh mineral oil placed inside the Thiele tube. From the bottom, heat was applied by means of a Bunsen burner and the temperature at which the drug melts is noted.[18,19]

Drug identification by FTIR Analysis: FTIR spectra of sample drug was recorded and the obtained peaks in the fingerprint region were compared with that of Diclofenac Sodium reference standard (B.P.) to confirm whether the taken sample is Diclofenac sodium.

Preparation of Phosphate buffer saline (pH 7.4): PBS pH 7.4 was chosen as the solvent medium to prepare the calibration curve of the drug as well as act as the release medium for drug release studies from the formulations as the pH of the synovial fluid has been measured to be ~7-7.5.[20,21] NaCl (8gm), KCl (0.2gm), Na_2HPO_4 (1.44gm) and KH_2PO_4 (0.24gm) were accurately weighed and mixed thoroughly until solubilization in 1000mL distilled water contained in a 1000mL volumetric flask.

Preparation of Standard Curve of Diclofenac Sodium: 100mg of sample drug was taken in 100mL volumetric flask. Then, make up the volume to 100mL with 100mL PBS. This was marked as 1st stock. From the 1st stock, 10mL was taken and made up to 100mL with PBS. This was marked as 2nd stock. From the 2nd stock, 1, 2, 3, 4, 5, 6 and 7 mL were taken in separate volumetric flasks and made up to 10mL each with PBS to get concentrations- 10, 20, 30, 40, 50, 60, 70 µg/mL respectively. Absorbance of each was checked in UV Spectrophotometer and graph was plotted.

Wavelength selection: Accurately weighed 100mg of drug (Diclofenac Sodium) was dissolved in 100mL of Phosphate buffer pH 7.4. After necessary dilutions, the resulting solution was scanned in the 190-400nm UV region to obtain the wavelength maxima. This wavelength was chosen for further analysis.

Preparation of DS-loaded microspheres: The required formulations i.e. microspheres were prepared by two separate methods i.e. Thermal control and Ionotropic cross-linking.

Thermal control technique- DS-loaded EC microspheres were prepared using thermal control method at mass ratios of 1:1, 1:1.5 and 1:2 (DS:EC+$CaSO_4$). The method of preparation was developed with modifications from the technique used by Jalsenjak et.al. (1976).[22] 50mL cyclohexane was taken in a beaker which was placed over a magnetic stirrer at 400 RPM and temperature set at 50ºC. Specific quantity of the coating material, Ethyl cellulose, was added to the beaker and temperature was gradually increased to 70º and maintained for about 25-30 minutes. The core material to be encapsulated, Diclofenac Sodium, was then added to the beaker and temperature was further increased to 80ºC. After maintaining the same conditions for another 60 minutes, the system was allowed to cool down to room temperature along with continued stirring. The microspheres were collected by filtration and washed with n-hexane three times. The microspheres were dried in a hot air oven at 40ºC for a period of 4 hours and stored.

Incorporation of $CaSO_4$ hemihydrate (12.5%, 25%, 50% and 75% of total polymer quantity) was carried out in certain formulations to obtain self-hardening microspheres. $CaSO_4$ was added at the same time polymer was added to the solvent system during formulation.

Formulations F-1 through F-15 were prepared by this technique.

Ionotropic cross-linking method- DS-loaded NaCMC microspheres were prepared by ionotropic cross-linking method with mass ratios of 1:1 (DS:NaCMC) and 1:1 (DS:NaCMC+$CaSO_4$). Under this technique of preparation of microspheres, there occurs a cross-linking of polyelectrolytes in the presence of counter-ions in the solution. This results in the formation of microspheres.[23,24] 2-3% Sodium alginate solution was prepared in a beaker and heated gently along with stirring. Suitable amounts of polymer (Na-CMC), drug and $CaSO_4$ were added to the above solution. The resulting mixture was injected through a 24 gauge syringe into 10% $CaCl_2$ solution. Microspheres were collected on a filter paper, were washed with distilled water two to three times and dried in a hot air oven at 40ºC for 4 hours. Formulations F-16 and F-17 were prepared by this method.

Characterization of the prepared microspheres:

Determination of Drug Encapsulation Efficiency and Loading Capacity-[25,26]

Accurately weighed 100mg of microspheres were suspended in 10ml PBS. The suspension was sonicated for 2-3 minutes and microspheres were isolated by filtration through Whatman filter paper. The filtrate was analysed for drug content after suitable dilution which indicated the free untrapped drug.

$$\text{Encapsulation efficiency \%} = \frac{(\text{Total drug added} - \text{free untrapped drug})}{\text{Total drug added}} \text{ X } 100$$

$$\text{Loading Capacity \%} = \frac{\text{Total entrapped drug}}{\text{Total weight of microspheres}} \text{ X } 100$$

Determination of Percentage Yield-[27]

% yield of microspheres was calculated by dividing total weight of the formulation by the total weight of non-volatile excipients used in the preparation of the formulation and is expressed as:

$$\text{Yield \%} = \frac{\text{Actual weight of microspheres}}{\text{Total weight of excipients}} \text{ X } 100$$

Particle size analysis-

Particle size of the prepared microspheres was determined by optical microscopy method. The size of microspheres was measured by a calibrated ocular and stage micrometer. 100 microspheres from one formulation of each batch (containing 0%, 12.5%, 25%, 50% and 75% $CaSO_4$) were taken at random and analyzed for their size and particle size distribution was plotted.[28]

Loss of Moisture- DS-loaded microspheres with varying quantities of polymers and ingredients were subjected to moisture loss studies which provide an insight on the hydrophilic nature of the microspheres. 500mg of prepared microspheres were weighed accurately and then stored in a desiccator at 37ºC for 48 hours containing fused calcium chloride which is hygroscopic in nature and as such has the tendency to readily absorb moisture from the surroundings. Final weight was noted when no further change in weight of sample was observed.[29]

$$\text{Moisture loss \%} = \frac{\text{Initial weight} - \text{Final weight}}{\text{Initial weight}} \text{ X } 100$$

Micromeritic properties-

The prepared formulations were also evaluated for their micromeritic properties such as- Angle of Repose, bulk density, tapped density, Carr's index and Hausner's ratio.

Angle of repose- It can be defined as the steepest angle at which a sloping surface formed of loose material is stable. It is indicative of inter-particular and cohesive forces as well as the flow properties of the material being investigated. Angle of repose of the formulated microspheres was measured by the fixed funnel method. The microspheres were poured through the funnel onto a stable base such that a heap of particular height and diameter is formed.[30] The angle of repose was determined by measuring the height and diameter of the heap of powder and calculated from the equation-

$$\text{Angle of Repose, } \theta = \tan^{-1}\left(\frac{\text{height}}{\text{radius}}\right)$$

The obtained angle is then compared and checked against the Carr classification of flowability of powder based on repose angle to understand the flow properties of the microspheres-[31]

Bulk and tapped density- Bulk and tapped density of formulation was found out using a 10ml graduated cylinder. Formulation was poured into the cylinder and the volume occupied was noted. Then, the cylinder containing the sample was tapped 100 times using the bulk density apparatus and the final volume was noted. Bulk and tapped density were calculated using the formula-[32]

$$\text{Bulk density} = \frac{\text{Mass of sample}}{\text{Volume occupied before tapping}}$$

$$\text{Tapped density} = \frac{\text{Mass of sample}}{\text{Volume occupied after tapping}}$$

Carr's index- also called compressibility index, it was calculated by the following formula.

$$\text{Carr's index} = \left(\frac{\text{Tapped density} - \text{bulk density}}{\text{Tapped density}}\right) \text{X } 100$$

Hausner's ratio- Hausner's ratio of formulations was calculated by comparing the tapped density with bulk density using the equation.

$$\text{Hausner's ratio} = \frac{\text{Tapped density}}{\text{Bulk density}}$$

The calculated Carr's index and Hausner's ratio are then compared and checked against the standard values.

In-vitro Buoyancy study- This test is done to check the floating properties of the formulated microspheres. Microspheres are spread over the surface of the release medium and are allowed to float at the surface or settle down to the bottom during a specific time period under constant agitation. At the end of the test period, the floating and settled microspheres are recovered separately, dried and weighed.[33]

$$\text{Buoyancy \%} = \left(\frac{\text{weight of floating microspheres}}{\text{weight of floating+settled microspheres}}\right) \text{X } 100$$

FTIR Spectrophotometric analysis-

The FTIR spectra of pure drug, drug with polymers (Ethyl cellulose, Sodium Carboxymethyl cellulose) and prepared formulations were recorded to study compatibility and any possible interactions between the drug and other excipients.

Surface morphology-

The surface characteristics of the microspheres were observed by SEM.

In-vitro biodegradability studies-

This test was carried out to gather knowledge of the degradation pattern of the formulated microspheres. 500mg microspheres were added to a sufficient quantity of PBS and placed in an incubator at 37ºC. Every three days collect the

microspheres by filtration and dry them completely by placing them in a hot air oven at 40º-45ºC for 3-4 hours. Weigh the dried microspheres. The differences in weight was checked by comparing it to the previously noted weight. After weighing place the microspheres in fresh PBS buffer and repeat the process every three days.[34]

In-vitro Drug release studies-

Formulations were analysed for their drug release properties by taking 100mg drug equivalent weight of formulation in a 15mL centrifuge tube and adding 10mL pH 7.4 phosphate buffer. The centrifuge tube was placed in an incubator with temperature conditions set at 37º±2ºC. 7mL samples were taken at regular intervals (3, 24, 48, 72, 96 hours and so on) and same amount of fresh buffer was introduced to maintain sink conditions. The withdrawn samples were suitably diluted and analysed by UV Spectrophotometer.

Kinetic modelling of drug release-

The drug release kinetics were studied using various models such as zero-order, first-order, Higuchi, Korsmeyer-Peppas and Hixon-Crowell. To study the release kinetics, data obtained from *in-vitro* drug release studies were plotted in the models. The best fit model was confirmed by the value of correlation coefficient near to 1. If n value is 0.45 or less, the release mechanism follows "Fickian diffusion" and higher values of 0.45 to 0.89 for mass transfer follow a non-fickian model (anomalous transport). The drug release follows Higuchi model of drug release and case II transport if the n value is 0.89. For the values of n higher than 0.89, the mechanism of drug release is regarded as super case II transport.

RESULTS AND DISCUSSION

Solubility studies of drug-It was carried out to test solubility of the drug in different solvents as well as to test its solubility in the dissolution medium (PBS pH 7.4) to be used. 10mL solvent was taken in a test tube and to it an excess amount of drug sample was added. The test tube was subjected to slight agitation and the solubility was checked. Sample of Diclofenac sodium taken was found to be highly soluble in methanol and acetone, freely soluble in ethanol and sparingly soluble in water, PBS buffer and cyclohexane.

Melting point of drug- After performing the melting point study using Thiele tube method, the melting point of the sample drug was noted to be 286.5ºC.

Drug identification-

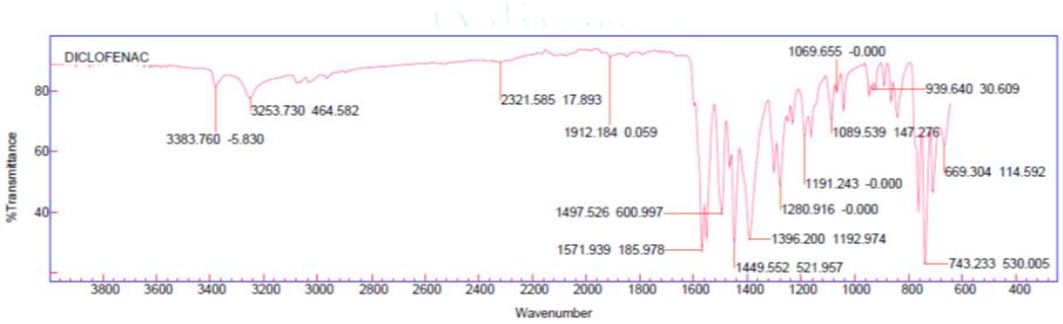

Fig 1: FTIR Spectra of sample drug

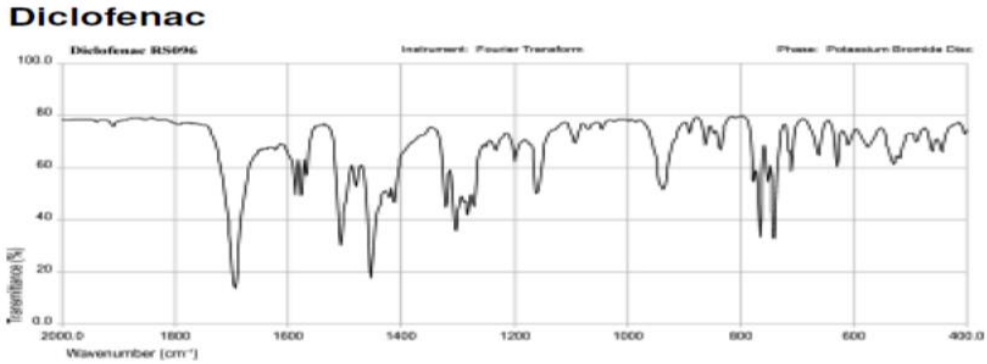

Fig 2: FTIR Spectra of Diclofenac Sodium reference standard (*British Pharmacopoeia*)

Comparing the fingerprint region of Diclofenac RS (British Pharmacopoeia) with that of the sample showed that the peaks obtained are similar. Hence, the sample used was identified to be Diclofenac Sodium.

Standard curve of Drug:

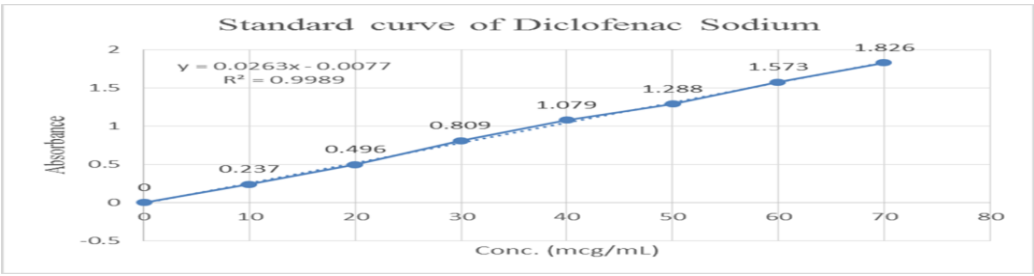

Fig 3: Standard curve of Diclofenac Sodium in pH 7.4 phosphate buffer

Standard curve for Diclofenac Sodium shows linearity over the range of 10-70µg/mL with regression equation y=0.0263x-0.0077 and correlation co-efficient R^2 value found to be 0.9989. On the basis of the regression equation, concentration of drug in various samples were calculated during the drug release studies.

Wavelength selection: The wavelength maxima (λmax) was observed at 276nm and this wavelength was adopted for further absorbance measurement.

Preparation of DS-loaded microspheres: The two methods adopted for preparation of microspheres i.e. thermal control and ionotropic cross-linking were found to be good as indicated by the high yield of microspheres in F-1 through F-17. Higher yield was indicative of lesser wastage and high efficiency of the encapsulation process. Ethyl cellulose and Sodium Carboxymethyl cellulose were used as polymers in varying ratios to prepare different batches of microspheres. $CaSO_4$ hemihydrate was used as an additional excipient to enhance the hardening of the microspheres. In thermal control technique, temperature was varied from 50ºC to 80ºC during different steps in the process to facilitate solubilisation of the polymer in the solvent as well as promote encapsulation of drug by the polymer. The prepared formulations were washed adequately with n-hexane to remove any traces of cyclohexane. Ionotropic cross-linking method was used to prepare two successful microsphere formulations (F-16 and F-17). Stirring rate and height of injection were varied to obtain smaller particle size of formulation.

The ingredients used for the preparation of microspheres and their respective quantities are depicted in Table 1

.

Table 1: Formulation ingredients and respective quantities used for the preparation of microspheres

Formulation	Diclofenac Sodium	Ethyl Cellulose	Sodium CMC	Calcium Sulfate	Cyclohexane	Sodium Alginate	$CaCl_2$
F-1	500mg	500mg	-	-	50mL	-	-
F-2	500mg	750mg	-	-	50mL	-	-
F-3	500mg	1000mg	-	-	50mL	-	-
F-4	500mg	437.5mg	-	62.5mg	50mL	-	-
F-5	500mg	656.25mg	-	93.75mg	50mL	-	-
F-6	500mg	875mg	-	125mg	50mL	-	-
F-7	500mg	375mg	-	125mg	50mL	-	-
F-8	500mg	562.5mg	-	187.5mg	50mL	-	-
F-9	500mg	750mg	-	250mg	50mL	-	-
F-10	500mg	250mg	-	250mg	50mL	-	-
F-11	500mg	375mg	-	375mg	50mL	-	-
F-12	500mg	500mg	-	500mg	50mL	-	-
F-13	500mg	125mg	-	375mg	50mL	-	-
F-14	500mg	187.5mg	-	562.5mg	50mL	-	-
F-15	500mg	250mg	-	750mg	50mL	-	-
F-16	500mg	-	500mg	-	-	1.125gm	10gm
F-17	500mg	-	437.5mg	62.5mg	-	1.125gm	10gm

Characterization of the prepared microspheres:

Determination of drug encapsulation efficiency and loading capacity-

Phosphate buffer pH 7.4 was used as the solvent to determine the drug encapsulation efficiency and drug loading capacity of the formulated microspheres. Encapsulation efficiency of the microspheres ranged from 67.88% to 90.24% with F-13 showing the lowest and F-5 showing the highest encapsulation. Encapsulation efficiency of the formulations were found to be high which might be due to the lesser solubility of the drug in cyclohexane and PBS. Drug loading capacity ranged from 22.94% to 61.41% as shown in Table 2. It was observed from the experiment that as the concentration of CaSO4 in formulation is increased its encapsulation efficiency is reduced i.e. higher concentration of CaSO4 results in lesser amount of drug being encapsulated and more amount of drug wastage during the encapsulation process. This could be the possible reason for loss of 10-30% of drug during the encapsulation process. Another explanation for loss of drug could be the presence of drug molecules on the surface of the formulated microspheres which when washed leads to drug removal leading to reduced % encapsulation in the microspheres.

Drug encapsulation efficiencies and loading capacities of the various formulations are given in Table 2.

Determination of percentage yield-

It was noted that the % yield for all the formulations was well over 50% which indicated the suitability of the methods used for the preparation of microspheres. Yield of microspheres ranged from 70.1% to 99.8% as demonstrated in Table 2. Determination of yield of each formulation was performed in a triplicate manner. These results also indicate that there was minimal loss of ingredients during the formulation process. It was also observed that whilst in some batches the % yield of microspheres increased with increase in polymer concentration, in others yield decreased with increase in polymer concentration. This variation can be attributed to the varying quantities of $CaSO_4$ used during the formulation of different batches.

Table 2: Drug encapsulation efficiency, Loading capacity and % Yield of the formulations

Formulation	EE%	DL%	Yield %
F-1	86.09	61.41	70.1
F-2	88.03	45.09	78.08
F-3	87.38	40.87	71.26
F-4	85.6	42.46	92.71
F-5	90.24	36.47	98.96
F-6	85.79	29.82	95.86
F-7	83.96	41.98	85.28
F-8	85.15	37.67	90.4
F-9	81.91	27.34	99.86
F-10	74.57	41.52	89.8
F-11	77.12	33.67	91.6
F-12	79.86	27.57	96.53
F-13	67.88	36.69	92.5
F-14	70.27	37.26	75.44
F-15	72.25	31.52	76.4
F-16	87.77	25.34	81.5
F-17	85.14	22.94	87.34

Particle size analysis-

Several formulation conditions such as- drug:polymer ratio, volume of the internal and external phase, stirring speed, height of injection etc. can be exploited to obtain microspheres of desired size range. According to a study by Saravanan et.al. (2011), smaller sized microspheres tend to get rapidly cleared from the joints after intra-articular administration. Additionally, larger microspheres show better sustained release properties than smaller microspheres due to the difference in surface area.[35] It has also been reported that larger sized particles have better retention properties.[8]

The average size of DS-loaded microspheres containing 0% (F-3), 12.5% (F-6), 25% (F-9), 50% (F-12), 75% (F-15) and F-17 were measured to be 50.52µm, 53.18µm, 55.71µm, 57.22µm, 56.49µm and 62.74µm respectively.

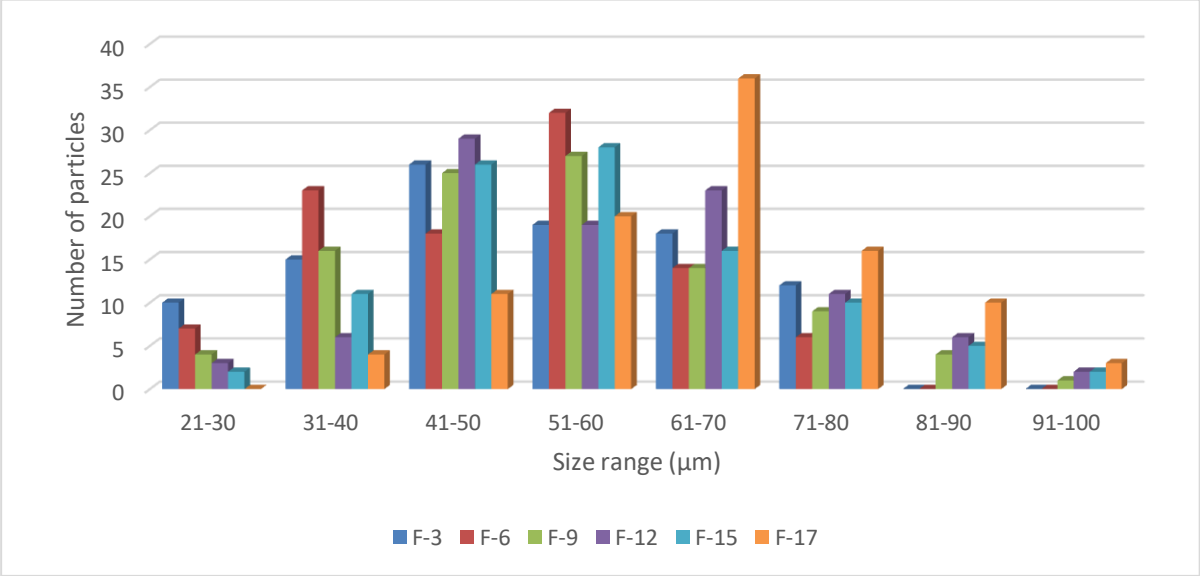

Fig 4: Particle size distribution of F-3, F-6, F-9, F-12, F-15, F-17

Loss of Moisture-

This evaluation of the prepared formulations was carried out under the specified conditions mentioned previously. After performance of the test and necessary calculations, it was noted that very small amounts of moisture were lost from the formulations which imply that the prepared microspheres contained negligible quantities of moisture. Moisture loss % was highest for F-13 i.e. 5.8% and lowest for F-6 and F-10 i.e. 1.2%. The results of moisture loss studies are given in Table 3

.

Table 3: % moisture loss from the formulations

Formulation	Initial Weight	Final Weight	% moisture loss
F-1	500	482	3.6
F-2	500	474	5.2
F-3	500	487	2.6
F-4	500	481	3.8
F-5	500	485	3
F-6	500	494	1.2
F-7	500	487	2.6
F-8	500	493	1.4
F-9	500	484	3.2
F-10	500	494	1.2
F-11	500	488	2.4
F-12	500	482	3.6
F-13	500	471	5.8
F-14	500	476	4.8
F-15	500	487	2.6
F-16	500	485	3
F-17	500	492	1.6

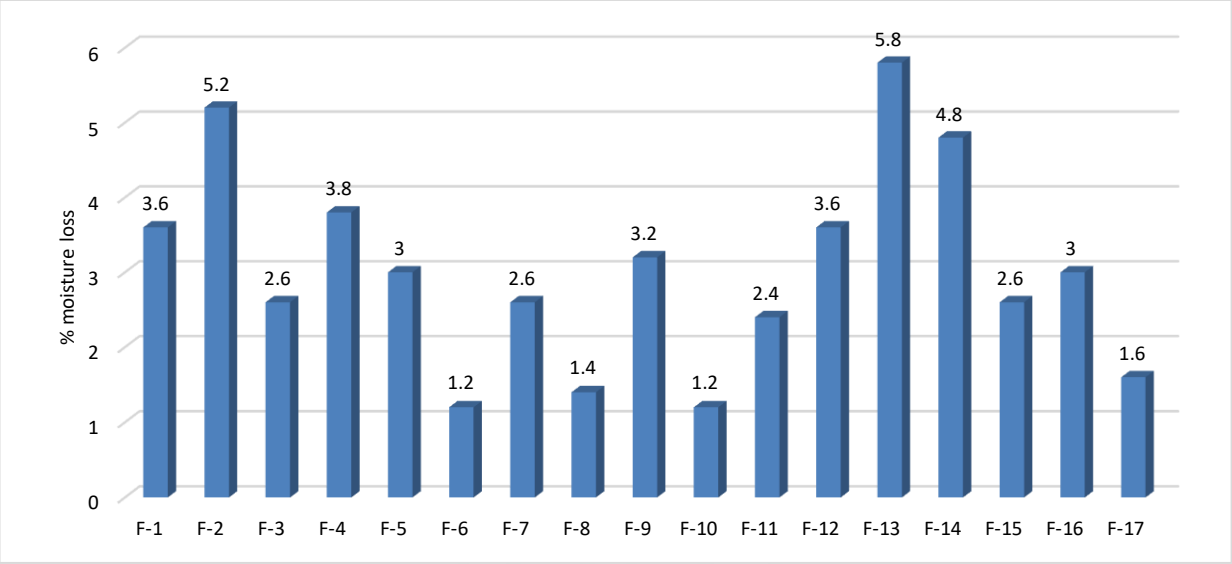

Fig 5: % moisture loss from the formulations

Micromeritic properties-

Table 4: Results of micromeritic properties analysis of the prepared microspheres

Formulation	Bulk Density	Tapped density	Carr's index	Hausner's ratio	Angle of repose	Flow Properties
F-1	0.333	0.378	11.9	1.13	34.99	Good
F-2	0.375	0.465	19.35	1.24	41.63	Fair
F-3	0.411	0.475	13.47	1.15	36.86	Good
F-4	0.427	0.473	9.72	1.1	25.55	Excellent
F-5	0.485	0.521	6.9	1.07	27.47	Excellent
F-6	0.533	0.575	7.3	1.08	26.56	Excellent
F-7	0.4	0.454	11.89	1.13	37.3	Good
F-8	0.434	0.48	9.58	1.11	20.55	Excellent
F-9	0.447	0.499	10.42	1.12	36.38	Good
F-10	0.39	0.472	17.37	1.21	43.36	Fair
F-11	0.424	0.467	9.21	1.1	24.62	Excellent
F-12	0.462	0.508	9.05	1.1	23.19	Excellent
F-13	0.362	0.411	11.92	1.14	36.38	Good
F-14	0.355	0.38	6.57	1.07	29.05	Excellent
F-15	0.449	0.509	11.78	1.13	37.87	Good
F-16	0.517	0.559	7.51	1.08	24.77	Excellent
F-17	0.482	0.545	11.55	1.131	36.47	Good

The results of micromeritic properties of the formulations are shown in Table 4. Bulk density of the various formulations ranged from 0.333 to 0.533gm/cm^3 while tapped density ranged from 0.378 to 0.575gm/cm^3. These values were satisfactory and indicated good packability of the formulations. From the calculations of bulk and tapped density, Carr's index and Hausner's ratio were derived which ranged from 6.57% to 19.35% and from 1.07 to 1.24 respectively. Formulation F-14 showed best compressibility property with a Carr's index of 6.57. Angle of repose of the formulations ranged from 20.55º-43.36º. All the formulations showed satisfactory micromeritic properties which indicated their good flow characteristics.

In-vitro Buoyancy study-

The floating behaviour of the various formulations were checked and it was found that % buoyancy of the microspheres ranged from 0% to 44% with F-17 showing no buoyancy at all and F-9 showing highest % buoyancy. Results of this test are depicted in Table 5.

Table 5: In-vitro % buoyancy of formulations

Formulation	Buoyancy %	Formulation	Buoyancy %
F-1	21	**F-10**	12.5
F-2	19.67	**F-11**	6.83
F-3	22.66	**F-12**	12.83
F-4	38.5	**F-13**	9
F-5	27.17	**F-14**	11.33
F-6	17.83	**F-15**	7.83
F-7	35.33	**F-16**	22.83
F-8	22.17	**F-17**	0
F-9	44		

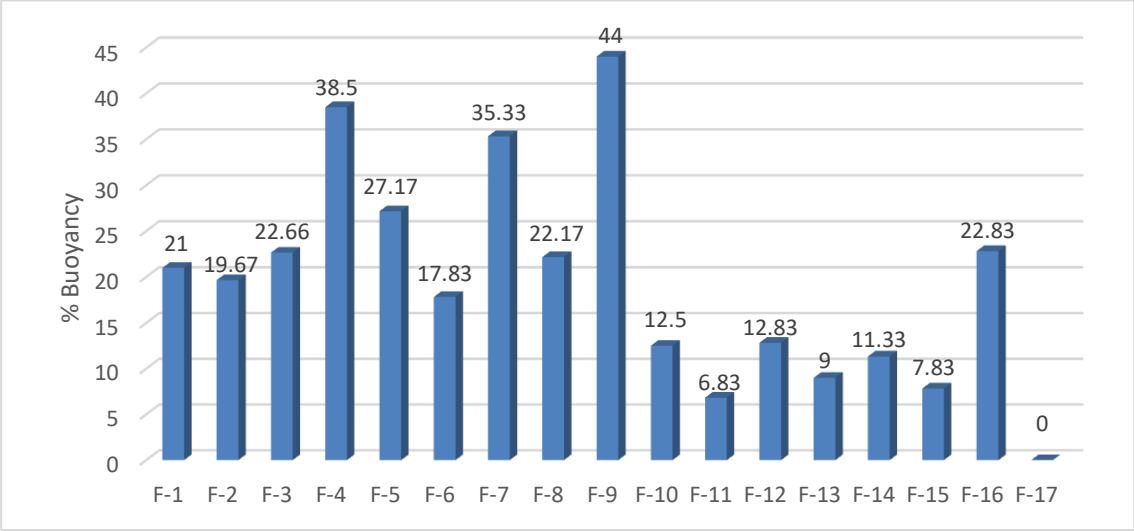

Fig 6: In-vitro % buoyancy of formulations

FTIR Spectrophotometric Analysis-

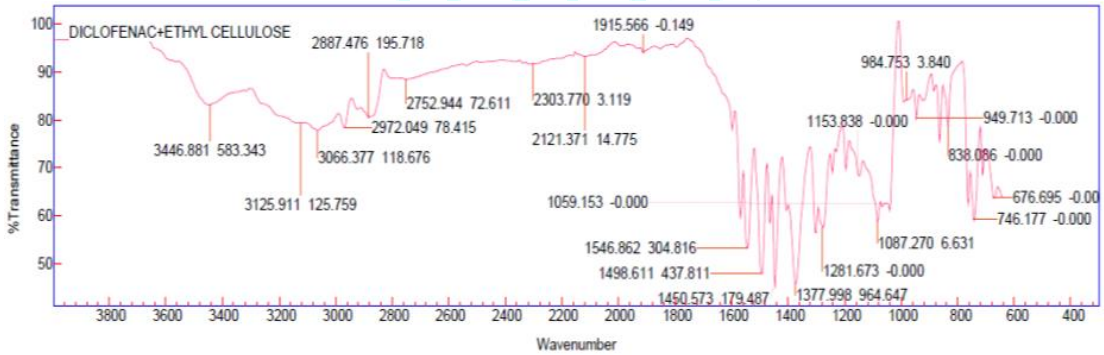

Fig 7: FTIR Spectra of DS + EC

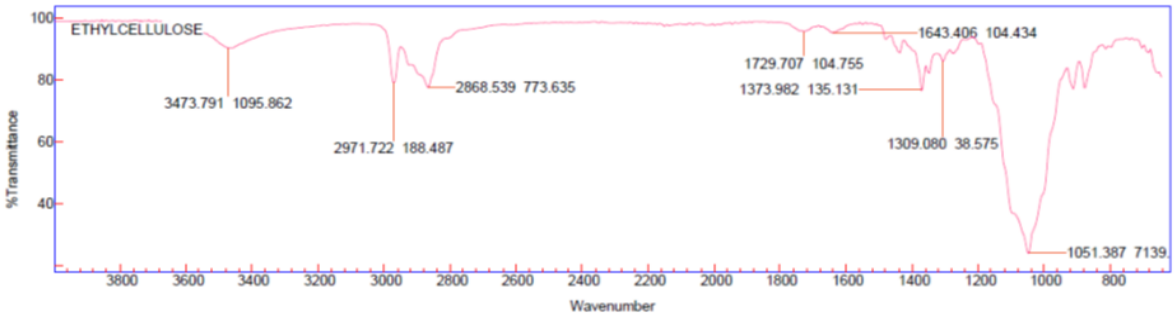

Fig 8: FTIR Spectra of EC

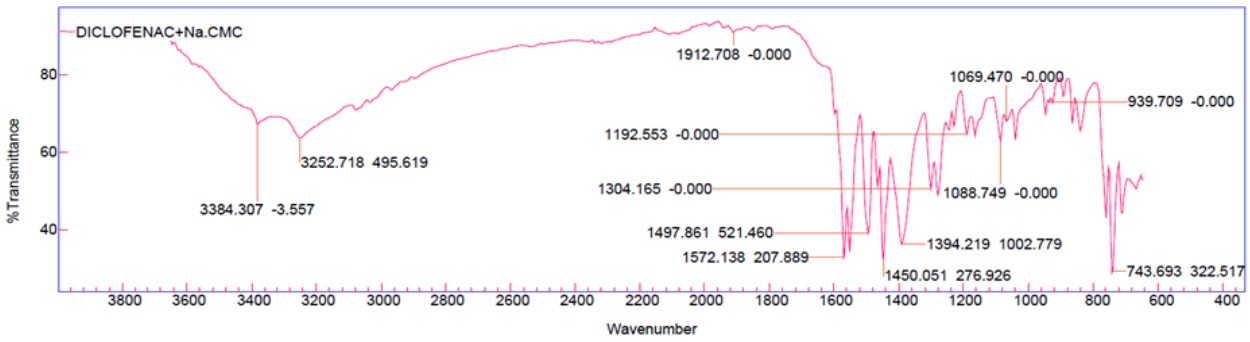

Fig 9: FTIR Spectra of DS + Na-CMC

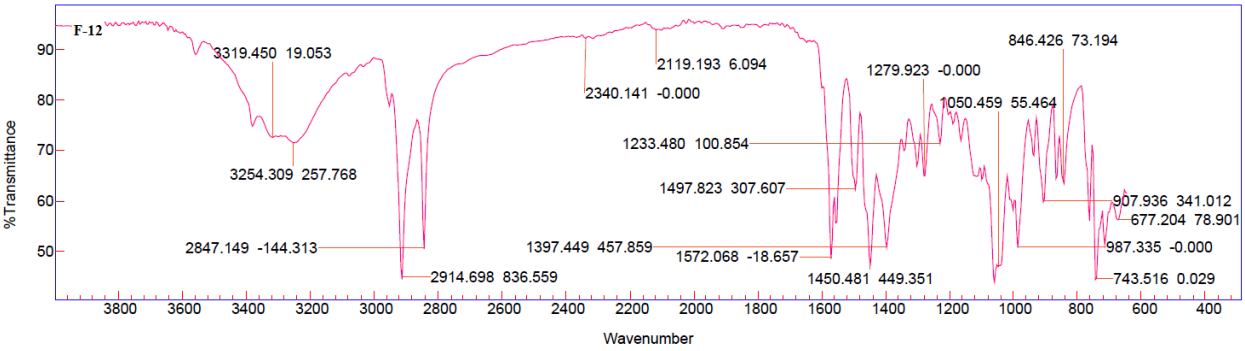

Fig 10: FTIR Spectra of F-12

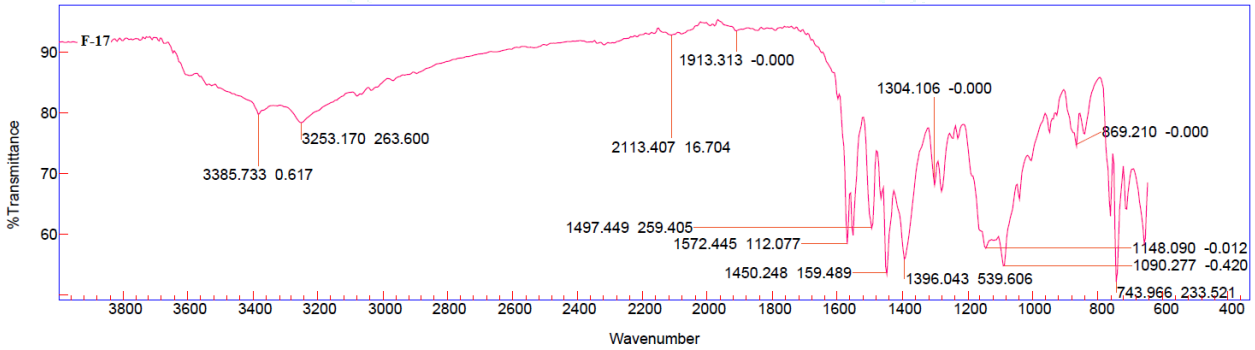

Fig11: FTIR Spectra of F-17

These recorded IR spectra were compared and analysed for their fingerprint region peaks and it was concluded that no major interactions took place between the drug and excipients with only minor changes in the peaks of functional groups.

Surface morphology-

The external surface morphology of two formulations of microspheres (F-15 and F-21) were studied using SEM. The surface of the microspheres can be observed to be rough due to the incorporation of higher concentration of drug during formulation. The roughness of the surface of the microspheres is also due to the use of $CaSO_4$ hemihydrate as a hardening agent during the formulation process. It can be inferred from the SEM images that the formulated microspheres were not porous and didn't contain any gaps on the surface.

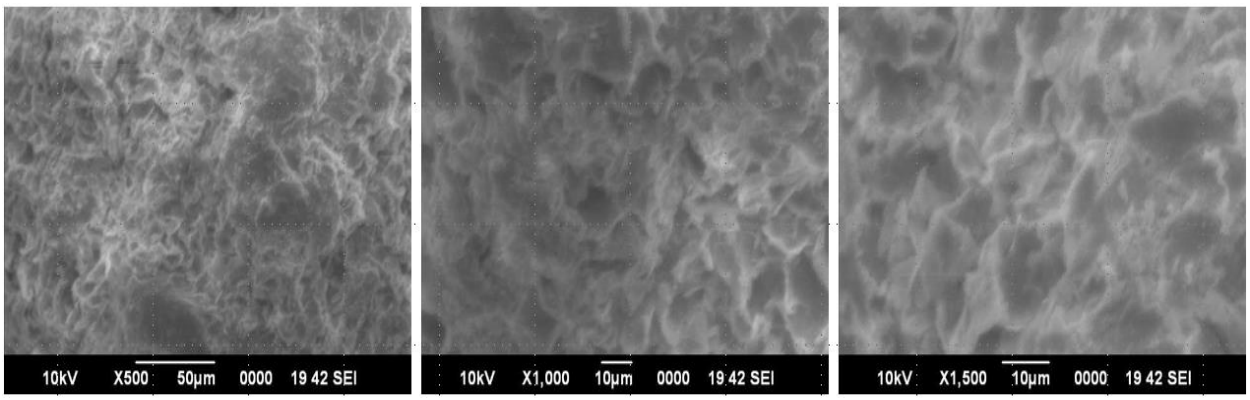

Fig 12: SEM photographs of DS-loaded microspheres (F-17)

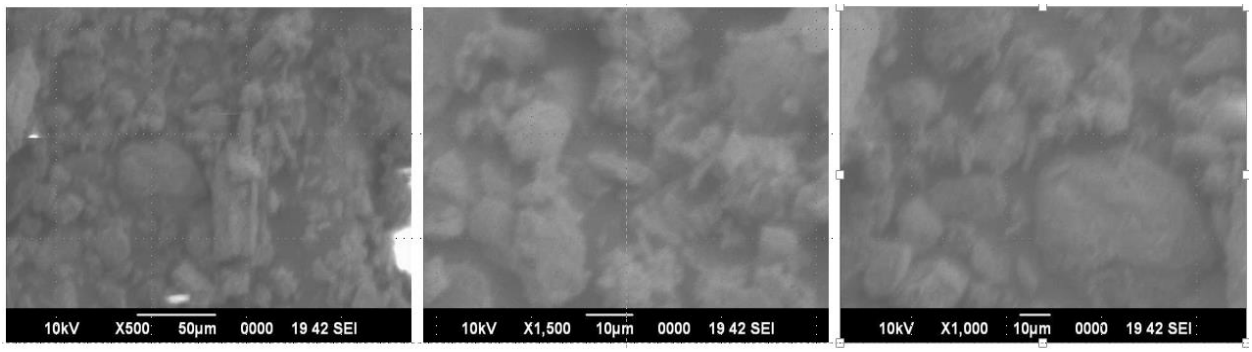

Fig 13: SEM photographs of DS-loaded microspheres (F-11)

In-vitro biodegradability studies- The prepared microspheres were subjected to biodegradation studies in-vitro using PBS as the medium over a period of 15 days during which the microspheres showed a constant decrease in weight which indicates that the microspheres gradually degraded over-time releasing the drug in an extended manner. The results of in-vitro biodegradability studies are shown in Table 6.

Table 6: In-vitro biodegradation pattern of formulated microspheres

Formulation	Day 0 (500mg)	Day 3 (mg)	Day 6 (mg)	Day 9 (mg)	Day 12 (mg)	Day 15 (mg)
F-1	500	384	252	143	35	0
F-2	500	407	284	168	62	18
F-3	500	412	334	210	106	33
F-4	500	416	328	216	93	16
F-5	500	424	320	206	124	52
F-6	500	436	359	242	158	36
F-7	500	438	354	259	142	23
F-8	500	445	367	275	188	45
F-9	500	431	375	263	167	30
F-10	500	457	388	293	175	68
F-11	500	438	363	287	172	56
F-12	500	455	391	306	192	81
F-13	500	415	338	263	184	86
F-14	500	436	371	286	189	57
F-15	500	442	375	298	215	129
F-16	500	381	278	174	82	16
F-17	500	405	291	184	117	42

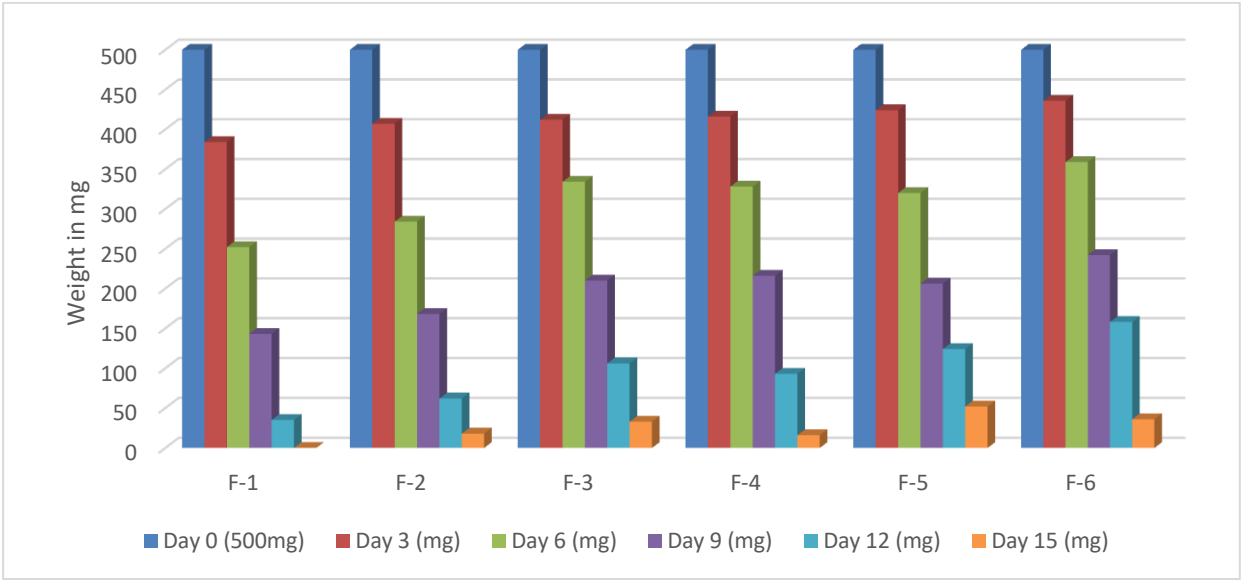

Fig 14: In-vitro biodegradation pattern of F-1 to F-6

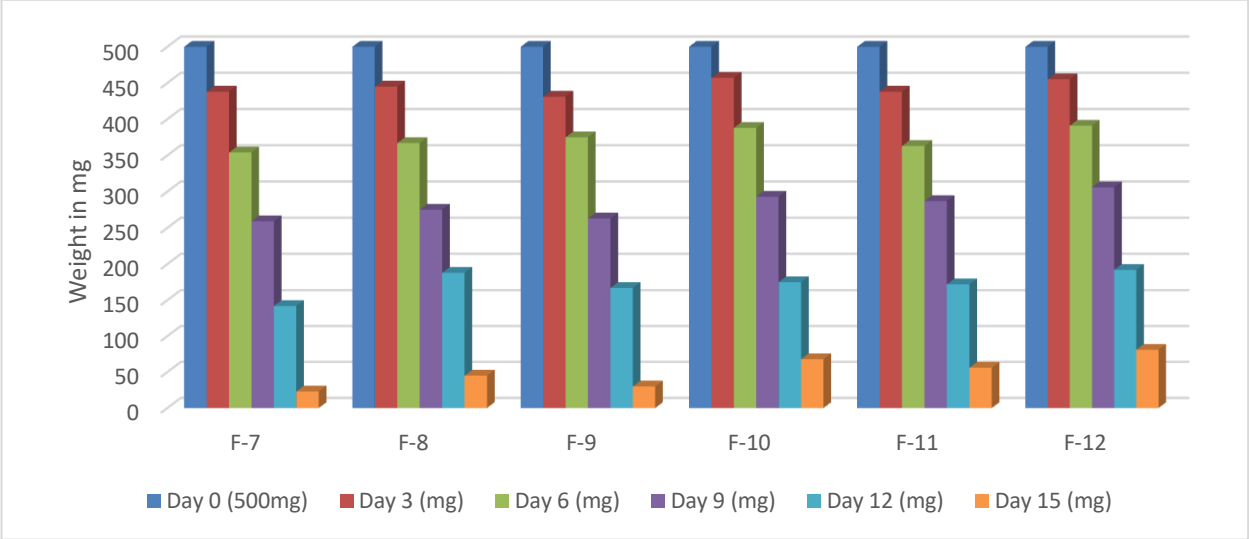

Fig 15: In-vitro biodegradation pattern of F-7 to F-12

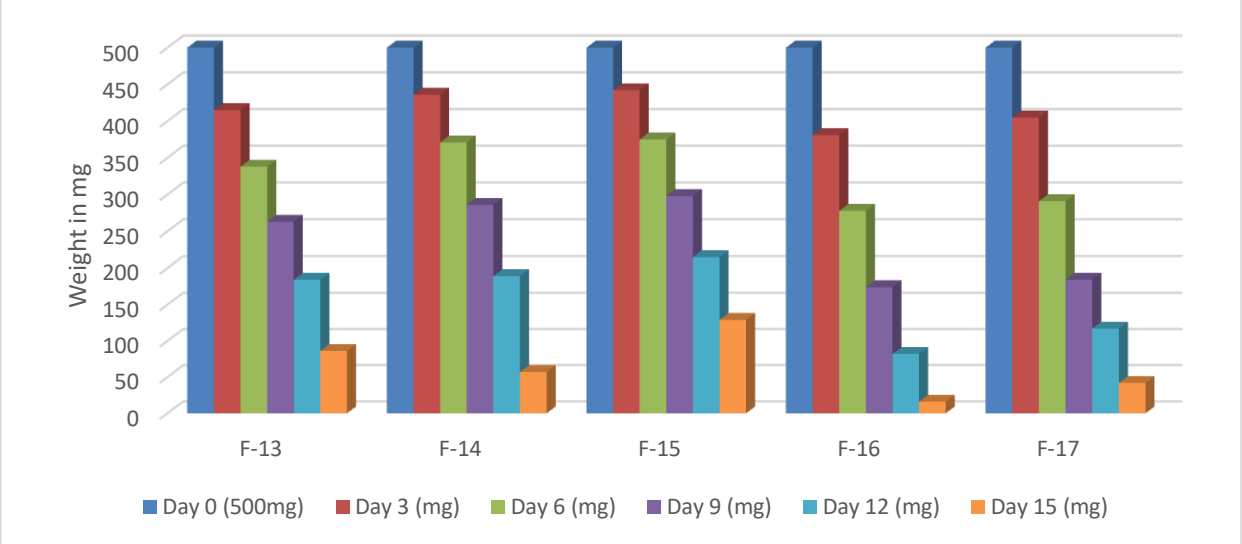

Fig 16: In-vitro biodegradation pattern of F-13 to F-17

In-vitro drug release studies-

The in-vitro drug release patterns of the various formulations are shown in figures. F-9 (12.5% $CaSO_4$) showed a prolonged release of drug for 11 days. At the end of day 5, microspheres of F-9 showed a drug release of nearly 65%. F-14 microspheres (containing 75% $CaSO_4$) released the drug at the fastest rate i.e. within 4 days. From the study of drug release pattern from the various formulations, it can be noted that the formulations incorporated with $CaSO_4$ tend to release drug for longer. The potential reason for this could be the hardening of the surface of the microspheres due to $CaSO_4$ which retards the release of the drug. However, higher concentration of $CaSO_4$ used for the preparation of microspheres allows lesser amount of drug to be encapsulated which is released within a few days.

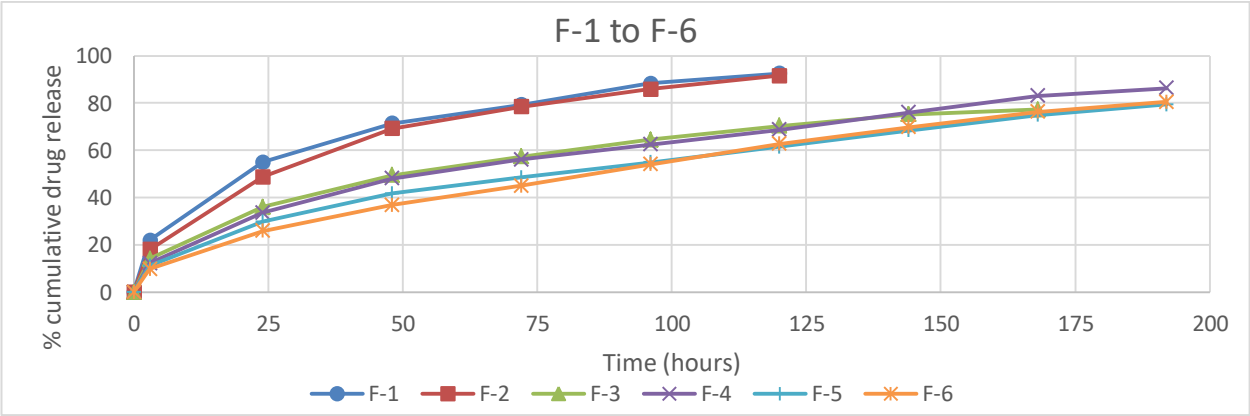

Fig 17: In-vitro drug release pattern of formulation F-1 to F-6

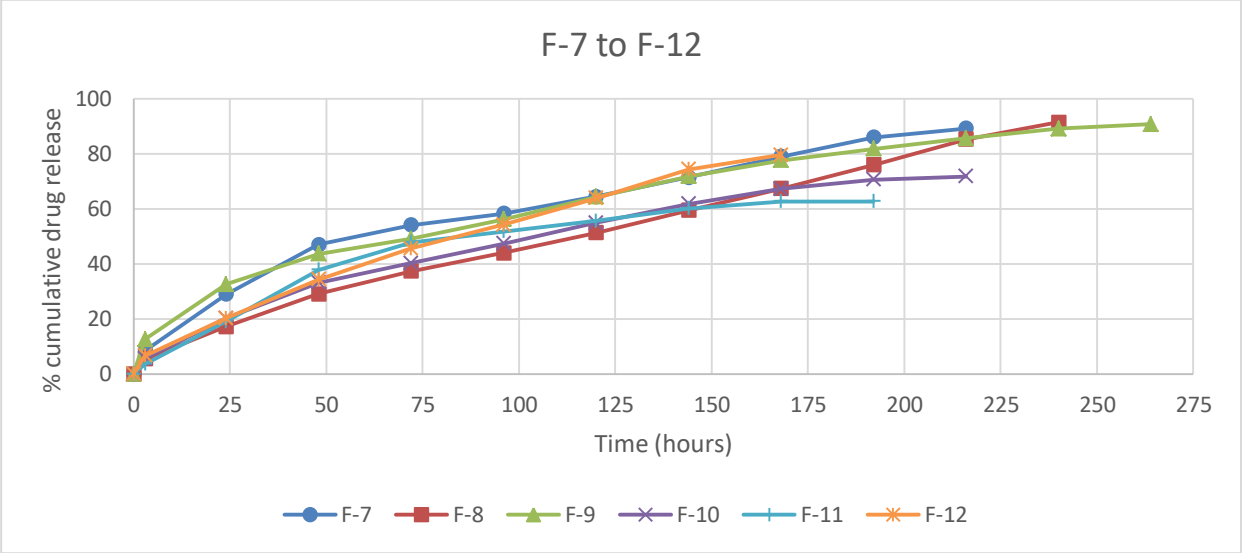

Fig 18: In-vitro drug release pattern of F-7 to F-12

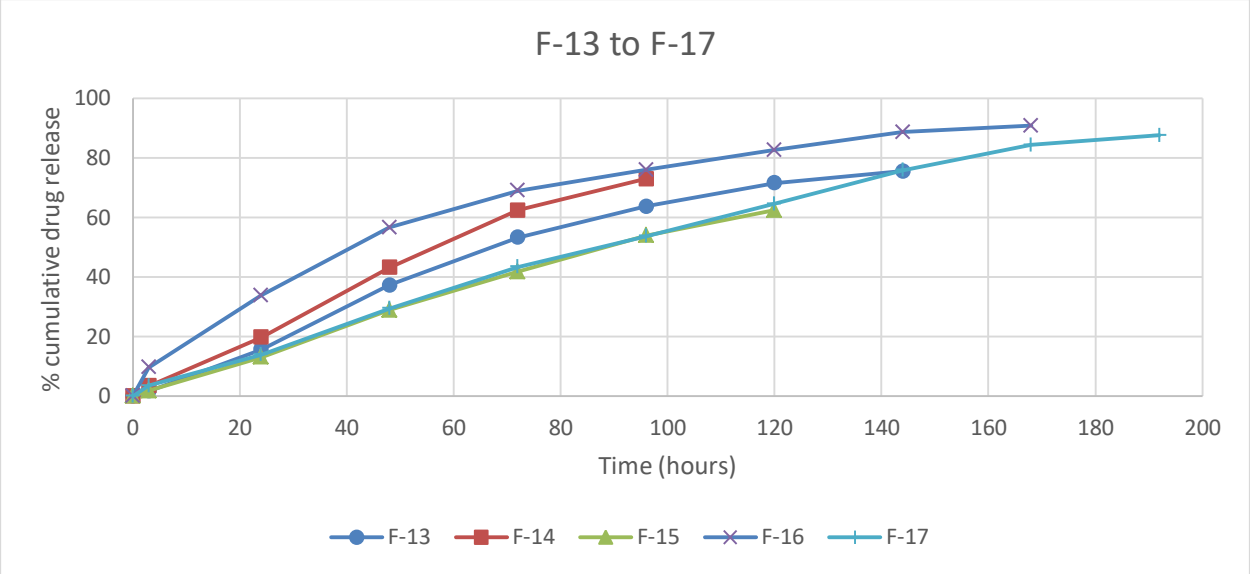

Fig 19: In-vitro drug release pattern of F-13 to F-17

Kinetic modelling of drug release-

Based on the tests performed on the different formulations of microspheres, F-9 was selected for kinetic modelling for drug release as it displayed satisfactory properties for all the tests as well as provided drug release the longest.

Fig 7: Kinetic modelling of drug release

Formulation	Zero-order (r^2)	First-order (r^2)	Higuchi (r^2)	Korsmeryer-Peppas	
				r^2	n
F-9	0.9085	0.9932	0.9961	0.8978	0.677

After fitting the drug release values to the various models, r^2 values of the zero-order and first-order model indicated that F-9 followed first-order drug release pattern. The highest regression (0.9961) was obtained for Higuchi model. Korsmeyer-peppas model was used to explain the mechanism of drug release. The value of slope (n) was calculated and found to be 0.677 which indicated that the drug release from the formulation was by non-fickian anomalous transport i.e. coupling of diffusion and erosion, which indicates that the drug release is sustained by more than one process.

CONCLUSION

The present study represents the first step in the development of Diclofenac sodium-loaded microspheres dedicated to intra-articular drug delivery. DS-loaded Ethyl cellulose and Sodium Carboxymethyl cellulose microspheres were successfully prepared by using thermal control and ionotropic cross-linking methods. From compatibility

studies by FTIR, it was confirmed that no chemical change occurred in the entrapped drug. It was also noted that for hardening of the microspheres, the higher concentration of $CaSO_4$ used resulted in lower amount of drug being encapsulated. Concentration of $CaSO_4$ also influenced drug release; formulations prepared using lower concentrations of the hardening agent (12.5%, 25%) provided drug release for longer than the formulations with higher concentrations (50%, 75%) and the formulations containing no hardening agent. After analysing the reports from the various tests carried out, it can be concluded that F-7, F-8 and F-9 show the most acceptable and satisfactory properties among which F-9 provides prolonged release the longest i.e. up to 11 days.

The prepared microspheres prolong the release of Diclofenac sodium which is potentially reproducible in other drugs (intended for pain and inflammation management in the bone joint) which exhibit rapid release rates.

REFERENCES

1. Kidd BL, Langford RM, Wodehouse T. Arthritis and pain. Current approaches in the treatment of arthritic pain. Arthritis research & therapy. 2007 Jun; 9(3):214.
2. Conaghan PG, Cook AD, Hamilton JA, Tak PP. Therapeutic options for targeting inflammatory osteoarthritis pain. Nature Reviews Rheumatology. 2019 May 8:1.
3. Walsh DA, McWilliams DF. Mechanisms, impact and management of pain in rheumatoid arthritis. Nature Reviews Rheumatology. 2014 Oct; 10(10):581.
4. Frank RG, Kashani JH, Parker JC, Beck NC, Brownlee-Duffeck MA, Elliott TR, Haut AE, Atwood CA, Smith EL, Kay DR. Antidepressant analgesia in rheumatoid arthritis. j Rheumatol. 1988 Nov 1; 15(11):1632-8.
5. Meng Z, Huang R. Topical treatment of degenerative knee osteoarthritis. The American journal of the medical sciences. 2018 Jan 1; 355(1):6-12.
6. Bisicchia S, Tudisco C. Hyaluronic acid vs corticosteroids in symptomatic knee osteoarthritis: a mini-review of the literature. Clinical Cases in Mineral and Bone Metabolism. 2017 May; 14(2):182.
7. van Laar M, Pergolizzi Jr JV, Mellinghoff HU, Merchante IM, Nalamachu S, O'Brien J, Perrot S, Raffa RB. Pain treatment in arthritis-related pain: beyond NSAIDs. The open rheumatology journal. 2012; 6:320.
8. Larsen C, Østergaard J, Larsen SW, Jensen H, Jacobsen S, Lindegaard C, Andersen PH. Intra-articular depot formulation principles: Role in the management of postoperative pain and arthritic disorders. Journal of pharmaceutical sciences. 2008 Nov 1; 97(11):4622-54.
9. Evans CH, Kraus VB, Setton LA. Progress in intra-articular therapy. Nature Reviews Rheumatology. 2014 Jan; 10(1):11.
10. Elmowafy M, Elrehaily A, Elharby A, Ali T, Elelian A, Elsemaihy A, Elgabry N. Transdermal Drug Delivery Systems; Review Article.
11. Buchholz HW, Elson RA, Engelbrecht E, Lodenkamper H, Rottger J, Siegel A. Management of deep infection of total hip replacement. The Journal of bone and joint surgery. British volume. 1981 Aug; 63(3):342-53.
12. Lyles KW, Siris ES, Singer FR, Meunier PJ. A clinical approach to diagnosis and management of Paget's disease of bone. Journal of Bone and Mineral Research. 2001 Aug; 16(8):1379-87.
13. Kim KK, Pack DW. Microspheres for drug delivery In BioMEMS and biomedical nanotechnology 2006 (pp. 19-50). Springer, Boston, MA.
14. Shahzad MK, Ubaid M, Raza M, Murtaza G. The formulation of flurbiprofen loaded microspheres using hydroxypropylmethycellulose and ethylcellulose. Advances in clinical and experimental medicine: official organ Wroclaw Medical University. 2013; 22(2):177-83.
15. Kondolot Solak E, Er A. pH-sensitive interpenetrating polymer network microspheres of poly (vinyl alcohol) and carboxymethyl cellulose for controlled release of the nonsteroidal anti-inflammatory drug ketorolac tromethamine. Artificial cells, nanomedicine, and biotechnology. 2016 Apr 2; 44(3):817-24.
16. Kulkarni S, Sharma SB, Agrawal A. PREFORMULATION-A FOUNDATION FOR FORMULATION DEVELOPMENT. International Journal of Pharmaceutical, Chemical & Biological Sciences. 2015 Apr 1; 5(2).
17. Larsson J. Methods for measurement of solubility and dissolution rate of sparingly soluble drugs.
18. http://www.chem.ucalgary.ca/courses/351/laboratory/meltingpoint.pdf (accessed on 17th May, 2019)
19. https://www.flinnsci.com/api/library/Download/46b1e79b05414c7f8e4164d9df5215ff (accessed on 17th May, 2019)
20. Cummings NA, Nordby GL. Measurement of synovial fluid pH in normal and arthritic knees. Arthritis & Rheumatism: Official Journal of the American College of Rheumatology. 1966 Feb;9(1):47-56.
21. Goldie I, Nachemson A. Synovial pH in Rheumatoid Knee-Joints I. The Effect of Synovectomy. Acta orthopaedica Scandinavica. 1969 Jan 1;40(5):634-41.
22. Jalsenjak I, Nicolaidou CF, Nixon JR. The in vitro dissolution of phenobarbitone sodium from ethyl cellulose microcapsules. Journal of Pharmacy and Pharmacology. 1976 Dec; 28(12):912-4.
23. Das MK, Senapati PC. Evaluation of furosemide-loaded alginate microspheres prepared by ionotropic external gelation technique. Acta Pol Pharm. 2007; 64(3):253-62.
24. Gangurde HH, Chavan NV, Mundada AS, Derle DV, Tamizharasi S. Biodegradable chitosan-based ambroxol hydrochloride microspheres: Effect of cross-linking agents. Journal of Young Pharmacists. 2011 Jan 1; 3(1):9-14.
25. Trivedi P, Verma AM, Garud N. Preparation and characterization of aceclofenac microspheres. Asian Journal of Pharmaceutics (AJP): Free full text articles from Asian J Pharm. 2014; 2(2).
26. https://www.sigmaaldrich.com/technical-documents/articles/materials-science/drug-delivery/drug-delivery-questions.html
27. Pandey N, Sah AN, Mahara K. Formulation and evaluation of floating microspheres of nateglinide. International Journal of Pharma Sciences aand Research. 2016; 7(11):453-64.
28. Pi C, Yuan J, Liu H, Zuo Y, Feng T, Zhan C, Wu J, Ye Y, Zhao L, Wei Y. In vitro and in vivo evaluation of curcumin loaded hollow microspheres prepared with ethyl cellulose and citric acid. International journal of biological macromolecules. 2018 Aug 1; 115:1046-54.
29. Rout PK, Nayak BS. Formulation design, preparation of losartan potassium microspheres by solvent evaporation method and it's in vitro characterization. Archives of Pharmaceutical Sciences and Research. 2009; 1(1):166-70.
30. Jagtap YM, Bhujbal RK, Ranade AN, Ranpise NS. Effect of various polymers concentrations on physicochemical properties of floating microspheres. Indian journal of pharmaceutical sciences. 2012 Nov; 74(6):512.
31. Riley RE, Hausner HH. Effect of particle size distribution on the friction in a powder mass. Int. J. Powder Met. 1970;6.
32. https://particle.dk/methods-analytical-laboratory/bulk-and-tapped-density/ (accessed on 19th May, 2019)
33. Andhariya JV, Burgess DJ. Recent advances in testing of microsphere drug delivery systems. Expert opinion on drug delivery. 2016 Apr 2; 13(4):593-608.
34. Ghosh S, Choudhury D, Roy T, Mamat AB, Masjuki HH, Pingguan-Murphy B. Tribological investigation of diamond-like carbon coated micro-dimpled surface under bovine serum and osteoarthritis oriented synovial fluid. Science and technology of advanced materials. 2015 May 14; 16(3):035002.
35. Saravanan M, Bhaskar K, Maharajan G, Pillai KS. Development of gelatin microspheres loaded with diclofenac sodium for intra-articular administration. Journal of drug targeting. 2011 Feb 1; 19(2):96-103.